The Review of DIABETIC STUDIES

REVIEW

Distal Sensorimotor Neuropathy: Improvements in Diagnosis

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Manuscript submitted April 17, 2015; resubmitted April 29, 2015; accepted April 30, 2015

■ Abstract

Neurological complications of diabetes are common, affecting up to 50% of people with diabetes. In these patients, diabetic sensorimotor neuropathy (DSPN) is by far the most frequent complication. Detecting DSPN has traditionally been a clinical exercise that is based on signs and symptoms. However, the appearance of morphometric and neurophysiological techniques along with composite scoring systems and new screening tools has induced a paradigm change in the detection and stratification of DSPN and our understanding of its natural history and etiopathogenesis. These newer techniques have provided further evidence that changes in small nerve fiber structure and function precede large fiber changes in diabetes. Although useful, the challenge for the use of these new techniques will be their sensitivity and specificity when widely adopted and ultimately, their ability to demonstrate improvement when pathogenic mechanisms are corrected. Concurrently, we have also witnessed an emergence of simpler screening tools or methods that are mainly aimed at quicker detection of large fiber neuropathy in the outpatient setting. In this review, we have focused on techniques and tools that receive particular attention in the current literature, their use in research and potential use in the clinical environment.

Keywords: diabetic neuropathy · small fiber neuropathy · axon reflex test · nerve conduction · nerve fiber density · laser Doppler imager flare · corneal confocal microscopy · electromyography · sudomotor function

1. Introduction

Diabetic neuropathy (DN) is arguably the most common complication of diabetes; it is also significant because of its associated morbidity and mortality [1, 2]. It is estimated that up to 50% of people with diabetes ultimately develop neuropathy; of these patients 50% are asymptomatic [1, 3]. Whilst acute diabetic neuropathies nearly always present with clear symptoms well recognizable by diabetes specialists, it is the gradually progressive neuropathy with silent onset that predominates, and is often noted only when it is well advanced. Also, acute diabetic neuropathies are associated with considerable morbidity, but gradually progressive neuropathies cause the bulk of the morbidity and mortality. It is now well understood that in the latter case there is a significant discordance between pathological severity and clinical features. However, because of the heterogeneous nature of the various diabetic neuropathies and the myriad of features, disease classification and characterization is difficult [4].

Length-dependent distal sensory neuropathy is the most common form accounting for approximately 80% of DN cases. It is associated with the greatest morbidity, mortality, and costs as it puts patients on a path towards loss of protective sensation, foot deformity, risk of injury, and infection. Ultimately, this path leads to foot ulceration, amputation, and death. The life expectancy of patients with neuropathic foot ulceration is approximately 50% at 5 years. This outcome is worse than many of the major cancers, including breast, colon, and prostate [5]. Eventually, distal sensory diabetic neuropathy can result in Charcot neuroarthropathy, a disabling and depressing chronic complication [3].

 DOI 10.1900/RDS.2015.12.29

In recent years, the development of modern investigation methods for nerve fiber structure and function has revealed pathological changes occurring prior to the development of symptoms or signs of neuropathy, in particular those in small nerve fibers [6]. These findings are challenging previously established classification systems and diagnostic algorithms. In this paper, we discuss the recent improvements in the field of diabetic neuropathy, with a specific focus on distal sensorimotor neuropathy (DSPN).

2. Definition and severity assessment of diabetic sensorimotor neuropathy (DSPN)

According to the classic concept by P. K. Thomas, diabetic neuropathy is a symmetric distal polyneuropathy with predominant sensory and relatively minor motor nerve involvement [7]. A statement by the American Diabetes Association in 2005 defined diabetic polyneuropathy as a clinical diagnosis based on the presence of symptoms and/or signs of peripheral neural dysfunction in people with diabetes after the exclusion of other causes (**Table 1**) [3]. In this classification, generalized symmetric polyneuropathy of diabetes was divided into three variants:

1. Chronic sensorimotor polyneuropathy
2. Acute sensory neuropathy
3. Autonomic neuropathy

However, these concepts do not include specific diagnostic criteria to confirm or exclude the diagnosis, nor do they provide criteria to determine severity. In 2005, The American Academy of Neurology developed a case definition and investigation protocol for distal symmetrical polyneuropathy, but the primary aim was to ensure future research studies to approach the question with greater consistency of case selection [8]. The authors concluded that the best approach to define DSPN would be an ordered set of definitions that include key features for the presence of neuropathic symptoms, ankle reflexes, distal sensation, muscle weakness/atrophy, and nerve conduction findings, and that are ranked by the likelihood of disease appearance [8]. According to this concept, an ordinal scale was developed that included 4 stages of DSPN probability, from highest ("++++") to lowest ("+"), with a recommendation to limit the enrolment of subjects into clinical research studies to those at the highest ordinal probability [8].

Abbreviations:
AAN – American Academy of Neurology
ADA – American Diabetes Association
ADPN – adiponectin
AKR1 B1 – aldo-keto reductase family member B1
AUC - area under the curve
CCM – in vivo corneal confocal microscopy
CHEPS – contact heat-evoked potentials
DFNS – German Research Network on Neuropathic Pain
DN – diabetic neuropathy
DNS – Diabetic Neuropathy Symptom Score
DSPN – diabetic sensorimotor neuropathy
ELMO1 – engulfment and cell motility 1
EMG – electromyography
ESC – electrochemical skin conductance
HRV - heart rate variability
IENFD – intraepidermal nerve fiber density
IpTT – Ipswich Touch Test
LDIflare – laser Doppler imager flare
LFN – large fiber neuropathy
MDNS – Michigan Diabetic Neuropathy Score
MF – 10 gm monofilament
MNCV – motor nerve conduction velocity
MNSI – Michigan Neuropathy Screening Instrument
mTCNS – modified Toronto Clinical Neuropathy Score
NCS – nerve conduction studies
NDS – Neuropathy Deficit Score
NeuPSIG – Neuropathic Pain Special Interest Group of the International Association for the Study of Pain
NICE – National Institute for Health and Care Excellence
NIS – Neuropathy Impairment Score
NSS-LL – Neuropathy Symptom Score of Lower Limbs
QLQ-CIPN20 – quality of life questionnaire to assess chemotherapy-induced peripheral neuropathy
QSART – quantitative sudomotor axon reflex test
QST – quantitative sensory tests
RR – interbeat
SCN9A – sodium channel, voltage-gated, type 9 alpha
SFN – small fiber neuropathy
SNAP – sensory nerve action potential
SNCV – sural nerve conduction velocity
SSR – sympathetic skin response
TCNS – Toronto Clinical Neuropathy Score
TRPA1 – transient receptor potential cation channel A1
UENS – Utah early neuropathy score
VGEF – vascular endothelial growth factor

2.1 Toronto consensus on the determination of diabetic neuropathy

The Toronto expert panel convened in 2009 to update and provide clear definitions and case characterizations of diabetic neuropathy [9]. They proposed separate definitions for typical diabetic polyneuropathy (i.e. the classic DSPN) and for atypical neuropathies. DSPN was defined as a symmetrical, length-dependent sensorimotor polyneuropathy attributable to metabolic and microvascular alterations resulting from chronic glycemic exposure and cardiovascular risk covariates.

Table 1. Symptoms, signs, and morphometry of distal sensimotor polyneuropathy (DSPN)

Symptoms	Signs	Neurophysiology/morphometry
Positive neuropathic sensory symptoms, including: - Numbness - Prickling - Burning, etc.	- Symmetric distal sensory loss to touch, vibration - Pinprick and thermal sensory loss and/or allodynia/hyperalgesia - Unequivocally decreased or absent ankle reflexes	- Abnormal nerve conduction studies (NCS) - Abnormal validated measure of small fiber neuropathy (SFN)

It was recommended that DSPN is classified into *i*) possible, *ii*) probable, and *iii*) confirmed DSPN, and they added a fourth category, *iv*) subclinical DSPN (**Table 2**) [9].

2.2 Severity assessment

If DSPN is confirmed once, its severity needs to be determined. Until the Toronto consensus, there were no agreed guidelines on the use of validated or objective tools to ascertain the severity of DSPN. The panel recommended the degree of nerve conduction abnormality as the minimal standard, but also supported an alternative approach suggested by Dyck in 1988. The latter approach grades the severity of DSPN from 0 to 2b, but does not take into account small fiber measures [9].

Assessment of severity using composite clinical scoring systems. The use of neuropathy composite scoring systems is one way of objectively measuring DSPN severity. Many of these systems have been developed and validated, but the following have found widespread application in both epidemiologic studies and clinical research:

- Neuropathy Deficit Score (NDS) of Boulton
- Michigan Neuropathy Screening Instrument (MNSI)
- Toronto Clinical neuropathy Score (TCNS)
- Diabetic Neuropathy Symptom Score (DNS)
- Neuropathy Impairment Score (NIS)
- Neuropathy Symptom Score of lower limbs (NSS-LL)
- Utah Early Neuropathy Score (UENS)

These systems are simple to administer, have very good concordance between operators when stratifying patients into different neuropathy severity levels, and may help provide an independent reference. Some of the newer scores have a reported ability to detect temporal change. The use of composite neuropathy scores is recommended in the Toronto consensus for measurement of DSPN severity [9].

Neuropathy Deficit (or Disability) Score (NDS). The NDS is a simple tool to grade the severity of neuropathy based on objective clinical examination assessing qualitative vibration perception, temperature differentiation, pinprick sensation, and presence of ankle reflexes [10-11]. No points are awarded for preserved sensation, but if impaired or absent 1 point is allocated per foot, except for ankle reflexes where 2 points are awarded if absent and 1 point if reflexes are present after reinforcement, thus giving a total of 10 points (**Table 3**). The following score system has been established:

0-2: clinical neuropathy is excluded
3-5: mild neuropathy
6-8: moderate neuropathy
>8: severe neuropathy

A score of >6 is also said to correlate well with a vibration perception threshold of >25 volts [11]. Many recent studies validating small fiber measures have used the NDS to denote the presence and stratify the severity of clinical neuropathy [12-13]. However, it must be noted that the NDS is different from the Neuropathy Disability Score devel-

Table 2. Diabetes-typical distal sensorimotor polyneuropathy (DSPN) according to the Toronto consensus definition [9]

Symptoms, signs, or laboratory findings	Recommended definition	Use
Presence of symptoms OR signs	*Possible* diabetic sensorimotor polyneuropathy	Clinical practice
Two or more symptoms OR signs	*Probable* diabetic sensorimotor polyneuropathy	Clinical practice
Any of symptoms OR signs AND abnormal neurophysiology/morphometry	*Confirmed* diabetic sensorimotor polyneuropathy	Clinical practice
No symptoms OR signs, BUT abnormal neurophysiology/morphometry	*Subclinical* diabetic sensorimotor polyneuropathy	Currently for research purposes only

oped by the Mayo Clinic, another validated method for DSPN assessment, with a reported sensitivity of 48% and a specificity of 91% [14-15].

Michigan neuropathy Screening Instrument (MNSI) and Michigan Diabetic Neuropathy Score (MDNS). MNSI consists of two separate assessments, a 15 point history questionnaire which is self-administered by the patient and physical assessments parameters of foot inspection, vibration perception assessment using a 128 Hz tuning fork, and monofilament testing [16]. Out of possible score of 8, scores >2.5 are considered to suggest DSPN [16]. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study used a standard of neurological examination evaluated against abnormal nerve conduction parameters. A combined MSNI value of >2.8 produced an area under the curve (AUC) of 0.81, with sensitivity of 43%, specificity 95%, positive predictive value 80%, and negative predictive value 80%, and explained a variance of r^2 = 27% [17]. It must be noted that the MNSI was developed primarily as a screening instrument. The Michigan Diabetic Neuropathy Score (MDNS) may be further used in those patients who have been screened positive with the MNSI for confirming DSPN; it has a neurological quantitative assessment component coupled with nerve conduction studies.

Toronto Clinical Neuropathy Score (TCNS). The TCNS was initially developed as part of a screening tool for diabetic neuropathy. It consists of three parameters:

1. Symptom scores (present = 1, absent = 0)
2. Reflex scores (present = 2, reduced = 1, absent = 0)
3. Sensory test scores (present = 1, absent = 0)

The possible maximum score is 19 (**Table 4**) [18]. Importantly, it remains one of the few scores that have been validated against morphometry, with a significant negative correlation with sural nerve fiber density (r^2 = -0.256, $p < 0.0001$). Subsequently, the authors published a modification, the mTCNS, to better capture the early sensory abnormalities of DSPN, and to eliminate muscle reflex tests which are notoriously variable between raters [19]. In a recent study comparing seven neuropathy composite scores in individuals with impaired glucose tolerance and symptomatic early neuropathy of less than 2 years' duration, mTCNS was the strongest discriminant, with an AUC of 0.99 ($p = 0.006$) for all subjects with neuropathy [20]. A cut off value of 3 had a sensitivity of 98% and specificity of 97%, with a positive predictive value of 99% and negative predictive value of 94% [20].

Table 3. Neuropathy Deficit (Disability) Score (NDS) [11]

Test	Value	Right foot	Left foot
Vibration perception threshold with 128 Hz tuning fork	Normal = 0 Absent = 1		
Temperature perception on dorsum of foot	Normal = 0 Absent = 1		
Pin prick proximal to hallux nail	Normal = 0 Absent = 1		
Ankle reflex	Present = 0 With reinforcement = 1 Absent = 2		
Total NDS out of 10		**/10**	

Neuropathy Impairment Score of the Lower Limb (NIS-LL) and NIS (LL)+7. The NIS-LL is a subset of the NIS; it allows objective assessment of the lower limbs, the region most commonly affected, and removal of variables not specific for DSPN. The NIS itself was an adaptation of the earlier Neuropathy Disability Score developed by Dyck and colleagues from the Mayo Clinic group by replacing those tests that are normal in DSPN [21-22]. The NIS-LL is complex to administer and quantifies by attributing a score ranging from '0' (no DSPN) to 88 (complete impairment). The components of the NIS-LL are sensation (vibration, pinprick, touch, pressure, joint position), muscle tendon reflexes (knee and ankle), and muscle group power assessments (hip flexion, hip extension, knee flexion, knee extension, ankle dorsiflexors, ankle planter flexors, toe extensors, toe flexors). In the Rochester Diabetic Neuropathy Study, Dyck and colleagues adapted the NIS-LL, adding in vibration detection thresholds, specific parameters from nerve conduction studies (NCS), and heart rate variability with breathing to yield the NIS(LL)+7 [4, 23]. A further advantage of the NIS(LL)+7 is its ability to assess dynamic and temporal changes in DSPN severity. For the Roch-

ester study cohort, the reported overall 2-year mean and standard deviation change in NIS(LL)+7 were 1.08 points and 3.57 points, respectively [23]. The authors also reported that the diabetes individuals worsened their NIS(LL)+7 by 0.34 points/year, while those with DSPN had their scores reduce by 0.85 points/year [23].

Table 4. Toronto Clinical Neuropathy Score by Bril and Perkins [18]

Symptom scores		**Reflex scores**		**Sensory test scores**	
Foot pain	Present = 1 Absent = 0	Knee reflexes	Present = 0 With reinforcement = 1 Absent = 2	Pinprick	Present = 1 Absent = 0
Numbness	Present = 1 Absent = 0	Ankle reflexes	Present = 0 With reinforcement = 1 Absent = 2	Temperature	Present = 1 Absent = 0
Tingling	Present = 1 Absent = 0			Light touch	Present = 1 Absent = 0
Weakness	Present = 1 Absent = 0			Vibration	Present = 1 Absent = 0
Ataxia	Present = 1 Absent = 0			Position sense	Present = 1 Absent = 0
Upper limb sensory symptoms	Present = 1 Absent = 0				
Subtotal	/6	/8			/5
Total	**/19**				

Utah Early Neuropathy Score (UENS). Most neuropathy composite scores assess reflexes, motor strength as well as sensory symptoms and signs. Therefore, they have a significant large fiber bias. This may result in a diminished sensitivity to early neuropathy which is predominantly related to sensory perception. The Utah Early Neuropathy Scale (UENS) was developed by a North American collaborative with the specific aim of detecting and quantifying early small fiber-mediated sensory neuropathy and to recognize modest changes in sensory severity [24]. Unusually, for a composite score, it was designed at the outset to allow detection of temporal changes in anatomical distribution of pin prick sensation [24]. Consequently, it puts more importance on cutaneous pin prick sensation and allodynia, conferring it 26 out of possible 42 points, and allocating only 16 points to large fiber modalities and motor examination. Accordingly, validation data demonstrated good correlation with sural sensory amplitude ($r = 0.40$, $p < 0.002$) and with intraepidermal nerve fiber density ($r = 0.43$, $p < 0.001$) [24]. However, it also correlated strongly with the MDNS ($r = 0.89$, $p < 0.0001$) and the NIS-LL ($r = 0.86$, $p < 0.0001$). When the criterion for DSPN was defined as symptoms of neuropathy, with confirmed abnormalities by two or more electrodiagnostic, electrophysiological, or histological tests, the UENS had a sensitivity of 92% (higher than 67% for MDNS and 81% for NIS-LL). It also had a specificity similar to other scores (unreported in the paper, but estimated around 75% from the ROC) and an AUC of 0.88 (MDNS 0.77 and NIS-LL 0.81) [24].

3. Diagnostic techniques for DSPN

As described previously, there is now increasing evidence to suggest that neuropathy of the smaller unmyelinated Aδ and C fibers may precede large fiber neuropathy, especially in type 2 diabetes and in states of impaired glucose tolerance [6, 25-28]. These fibers comprise 75-90% of the peripheral nerves, and mediate pain and temperature as well as autonomic function [27, 29]. Researchers are increasingly convinced that small fiber neuropathy (SFN) may be the 'microalbuminuric' equivalent of DSPN, and may have a potential future role in studies of putative agents aimed at treating diabetic neuropathy. This has led to an increased effort in developing techniques for accurate characterization of SFN since traditionally used clinical laboratory measures (such as NCS) do not identify the disease early enough, and the previously discussed composite scores are not specific for SFN. For the purpose of this review, we have divided the methods for investigating small fibers into those measuring small fiber structure and those measuring function (**Table 5**). Although current equipment gives objective, reproducible, and quantitative measures of large fiber function, NCS is only accessible in neurophysiology laboratories. Therefore, in practice it is only used when clinical pres-

Table 5. New techniques in distal sensorimotor polyneuropathy assessment

Small fiber measures
Small fiber structure
- Intraepidermal nerve fiber density measurement
- Corneal confocal microscopy
Small Fibre Function
- LDIflare method
- Neuropad™
- EZScan™ method/ Sudoscan®
- Thermal Threshold measurements
- Microneurography
Others
- Contact heat evoked potentials (CHEPS)
- Current perception thresholds
- Q-SART and Q-Sweat™
Large fiber measures
Neurometrix™ device
Vibratip™
Ipswich Touch Test (touch-the-toes test)
Nerve conduction studies (e.g. NC-stat®/DPNCheck™)

entation is atypical or in the context of a research study. Recently, portable devices such as the DPN-Stat™ device have added in a dimension of bedside accessibility to NCS; their utility is briefly discussed in section 3.3.

3.1 Small fiber neuropathy (SFN) - definition and classification

There is no agreed definition of small fiber neuropathy [30-31]. In the literature, definitions have reflected the various methods used by research teams, including physiological methods to asses small nerve fiber function (or dysfunction) and quantitative (but psychophysical) measures of thermal and pain thresholds and abnormalities on skin biopsy quantification [31]. Therefore, the presence of SFN is determined by centile cut-off values appropriate for age and gender, rather than by a clear clinical definition [30, 32-33].

The clinical hallmark of lower limb small fiber neuropathy is a disturbance in pain sensation. However, numerous studies have shown that disordered small fiber function and/or structure occurs early in the course of diabetes, even with impaired glucose tolerance, and can even be present in the absence of disordered pain sensation [34-35]. In patients with disordered pain sensation characteristic of SFN, nerve conduction tests are often normal, and have a supportive role to exclude differential etiologies such as chronic inflammatory demyelinating polyneuropathy [21, 31].

To date, there is no standardized classification of SFN, although the Toronto consensus includes abnormality in a validated measure of small fiber neuropathy in its classification of DSPN (**Tables 1 and 2**). Upcoming studies are demonstrating progression of SFN to higher degrees of neuropathy in diabetes, but evidence from more longitudinal studies is required to draw definite conclusions [35-36].

3.2 Methods for assessment of small fiber neuropathy

Intraepidermal nerve fiber density measurement (IENFD). Skin biopsy with measurement of intraepidermal nerve fiber density (IENFD) is now a widely accepted technique to detect SFN [30, 33]. The technique was initially described by McCarthy *et al.* in 1995, and applied to evaluate DSPN in non-diabetic sensory neuropathies [37]. Since then it has been shown to be an objective and reliable marker of SFN; indeed it is considered the gold standard in some guidelines [38]. The method requires a specimen that is obtained by a 3 mm punch biopsy of hairy skin at the distal leg usually 10 cm above the lateral malleolus in the region of the sural nerve. The specimen is immunostained for protein gene peptide 9.5 (PGP 9.5), a panaxonal neuronal antigen [39]. The bright field immunochemistry protocol by Lauria *et al.* is the most widely used. Quantification of linear IENFD results are expressed in number of fibers per millimeter in at least three sections of 50 μm [33, 39-40]. Another technique, indirect immunofluorescence with optical fluorescence or confocal microscopy, is also used [40]. The procedure is simple to perform and well-tolerated; healing occurs within 7-10 days. [29, 38]. The complication rate is low; the most common reported side effect is mild wound infection recoverable with topical antibiotic therapy [38]. Kennedy *et al.* reported a relationship between IENFD and DSPN severity in diabetic candidates awaiting pancreas transplantation compared with control subjects [41].

Operating characteristics of IENFD in detecting neuropathy are robust, sensitivity is between 60% and 95% and specificity between 90% and 95% [30-31, 42]. In the Lifestyle Intervention for Pre-Diabetic Neuropathy study, there was up to 1.4 ± 2.3 fibers/mm ($p < 0.004$) improvement in IENFD after 1 year of treatment, indicating that

this technique could detect and quantify cutaneous reinnervation [43]. The same group have recently demonstrated that in established diabetes a structured, supervised weekly exercise regime could lead to improvement in IENFD (1.5 ± 3.6 fibers/mm diabetic subjects vs. -0.1 ± 3.2 fibers/mm controls, p = 0.03) [44]. This improvement has also been shown in patients with metabolic syndrome [45], confirming that IENFD is an useful marker with potential to detect SFN reversal. However, these studies were uncontrolled. Hence, controlled trials are necessary to demonstrate efficacy and true effect.

An additional advantage is the availability of robust worldwide normative data and a standardized laboratory protocol [39]. The main drawback is its invasive nature and the requirement for a specialist laboratory background, as reported by many. Nevertheless, its demonstration of reversibility of small nerve fiber dysfunction nominates this technique as standard for investigation of new therapies to prevent or reverse early DSN.

In vivo corneal confocal microscopy (CCM). This has been shown to be a rapid, non-invasive ophthalmic technique that can accurately quantify corneal innervation in the human subbasal plexus [46]. In diabetes, it has been shown that reductions in corneal innervation occur early in the disease, are symmetrical between left and right eye, worsen with increasing severity of DSPN, and that such changes are parallel to similar changes in IEFND in the feet [47-49]. Tavakoli *et al*. from the Manchester group have demonstrated that all parameters measured in CCM correlate strongly with NDS and indeed with DPSN severity (corneal nerve fiber density r = -0.475, p < 0.0001; nerve branch density r = -0.511, p < 0.0001; nerve fiber length r = -0.581, p < 0.0001) [48]. Receiver operating characteristic curve analysis for the diagnosis of neuropathy (using NDS >3 to indicate clinical neuropathy) defined a sensitivity of 82% and specificity of 52%. If patients with foot ulcer risk were defined (NDS >6 as the standard), sensitivity increased to 71% and specificity to 64% [48].

Another recent study reported that corneal nerve fiber length was the best discriminator of the CCM variables, with an optimized sensitivity of 85% and specificity of 84% for identifying DSPN by using CCM [50]. They also reported on the advantage of separate thresholds to respectively identify or exclude DSPN. Whilst a single threshold offered clinically acceptable operating characteristics, separate thresholds may have more robust performance with sufficient predictive validity to identify individuals who are at risk of developing DSPN [50]. Using CCM variables, studies have documented improvement in terms of nerve fiber repair with tight glycemic control [51], 6 months after pancreas transplantation [52], after simultaneous kidney-pancreas transplantation [53], and with insulin pump therapy [54]. The manual counting method is time-consuming and costly, but newer automated methods have been validated successfully, with reported area under the curve for identifying DSPN of 0.82 for the manual method and 0.80 for the automated algorithm [55-57].

In a cohort of recently diagnosed subjects with type 2 diabetes (with a mean duration of diabetes of 2.1 ± 1.8 years), Ziegler *et al*. showed that CCM and IENFD were reduced below the 2.5th percentile in 21% and 14% of patients, respectively [58]. Surprisingly, there was poor concordance between those abnormal for CCM and IENFD, confirming that small nerve fiber structural change is patchy and heterogeneous in nature [58]. With more widespread research application of CCM, newer image acquisition algorithms are being generated; some encompassing larger scanning areas than the traditional image frames of 0.15 m^2 [28, 58-59].

Laser Doppler imager flare (LDIflare). Based on the observation that the neurogenic axon reflex-mediated flare response is abnormal in individuals with SFN, the size of the stimulated axon reflex flare has been proposed as a non-invasive measure of small fiber neuropathy [60]. When action potentials are generated in nerve endings of C-fibers or the Aδ fibers, they are conducted orthodromically and transmitted antidromically, exciting the adjacent neurons [61]. This results in the release of vasoactive neuropeptides such as calcitonin gene-related peptide, substance P, and histamine, provoking a vasodilatory flare response [61-62]. This response can be quantitated by determining the induced flare area and intensity using laser Doppler imaging.

In laboratory studies, using iontophoresis techniques, it has been shown that neurovascular vasodilation accounts for up to 30% of the total response to acetylcholine, and it is significantly reduced in DSPN [63]. The LDIflare technique is the clinical application of this principle to measure the induced flare area at the dorsal foot skin. It utilizes a scanning laser device and skin heating as the nociceptive stimulus [64]. As with IENFD and CCM, LDIflare has been validated against large

and small fiber markers and bears a strong correlation to IENFD ($r = 0.77$, $p < 0.001$) [62]. It can detect abnormal small fiber function in increasing severity of diabetic neuropathy [64-65], impaired glucose tolerance with normal thermal thresholds [26], and as reported recently, in non-diabetic non-neuropathic individuals with hypertriglyceridemia [66]. Studies have shown that glycemic control and HbA1c have a strong relationship with LDIflare results [67-68].

In the detection of clinical neuropathy, the LDIflare technique has a sensitivity of 70-75%, specificity of 66-85% [32, 68], positive predictive value of 74%, and negative predictive value of 86% [32], depending on the methodology used. The modified LDIflare technique employs a higher skin heating temperature, but for a shorter duration (47°C for 3 minutes V 44°C for 20 minutes), and can therefore be administered easily in a clinic setting [69]. It may also have a role in diagnosis and quantification of chemotherapy-induced peripheral neuropathy. Also, it correlates with the QLQ-CIPN20 symptom scores in those patients receiving platinum-based therapies ($r = 0.81$, $p = 0.001$) and taxane-based agents ($r = 0.58$, $p = 0.027$), when sural nerve conduction velocity and amplitude do not [70].

Sudomotor function assessments. Up to 56% of type 1 diabetes patients suffer from a reduction in active foot skin sweat glands, and up to 40% have a reduced sweat evaporation rate [71]. Abnormalities of C-fibers in DSPN leads to sudomotor dysfunction manifesting as a reduction in plantar sweating, plantar anhidrosis, and dry skin [72]. Sudomotor function tests provide information on peripheral autonomic function. In specialist centers, they are currently used as adjuvant or screening tests for DSPN [73]. The quantitative sudomotor axon reflex test (QSART) [74-75] and the commercially available Q-Sweat [76] have been in clinical application for more than a decade. However, these procedures require expensive equipment and need purpose-built lab space.

Detection of sympathetic skin response (SSR) in the eccrine sweat gland is another useful method, but also requires dedicated testing laboratories [77-78]. A study comparing QSART and SSR found similar rates of detection of approximately 50% in a group with DSPN defined by the presence of symmetrical distal sensory disturbances and absent Achilles tendon reflexes [75]. More recently, the development of simple techniques that can be reliably administered in a clinical setting has leveraged the methods in clinical application. In this review, we have focused on the recently introduced Neuropad® and Sudoscan® methodologies.

Neuropad®. Neuropad® (Trigocare International GmbH, Germany) is a simple bedside screening test for DSPN, providing a qualitative/categorical indication of sudomotor dysfunction. A plaster is adhered to the plantar surface of the forefoot for 10 minutes; it changes color from blue to pink as the impregnated anhydrous cobalt II compound comes in contact with foot skin sweat [79]. Response is determined as normal (no neuropathy) if the color completely changes to pink or abnormal (presence of neuropathy) for absent or incomplete patchy color change [79]. The Neuropad® abnormal patchy/absent response has been shown to have a 70-95%% sensitivity, 50-71% specificity [80-83], and 98% negative predictive value for both large and small fiber DSPN (using clinical examination as reference standard). For small fiber dysfunction, the values are 86%, 71%, and 93%, respectively [82].

In an effort to develop a more precise quantitative analysis of color change, researchers from Manchester University have developed the sudometrics image analysis algorithm that uses digital analysis of a Neuropad® photograph. It can quantify the Neuropad® response in a range from 0% to 100% instead of the established categorical value [84]. In a recent paper, the authors have reported that this method improved overall diagnostic efficacy of the Neuropad® in detecting DSPN, especially small fiber neuropathy and autonomic neuropathy [84]. In comparison to CCM parameters, which served as reference standard, sensitivity and specificity of the Neuropad® was 88% and 78%, respectively; it was 88% and 83%, respectively, when SNAP was used as standard [84]. Importantly, the visual nature of the Neuropad® may have an additional role in patient self-examination and education about DSPN [85-87].

Sudoscan™. Sudoscan™ (Impeto Medical, France) is an FDA approved device for the assessment of sudomotor function as a marker of DSPN severity. The principle is based on measuring the electrochemical skin conductance (ESC) between the chloride ions in the sweat of hands and feet which are placed on stainless steel-based plate electrodes of the machine. Results are expressed in µ-Siemens units [88-89]. A low-voltage current (<4 V) is applied through the electrodes, attracting chloride ions from the sweat glands by reverse ion-

tophoresis [89]. An earlier device utilizing the same principle, EZScan™, has shown promise in non-invasive screening for type 2 diabetes and impaired glucose tolerance [90-91]. Studies with Sudoscan have shown that diabetes patients with DSPN have significantly lower ESCs of feet and hands than those without clinical DSPN or healthy controls (56.3 ± 3 vs. 75.9 ± 5.5 and 84.4 ± 0.9, $p < 0.0001$ for feet and 51.9 ± 2.4 vs. 67.5 ± 4.3 and 73.1 ± 0.8, $p < 0.0001$ for hands) [89]. Furthermore, increasing NIS-LL scores were associated with decreasing ESC values [89]. It has a reported sensitivity of 77-78% and specificity of 67-92% for the detection of clinical DSPN [89, 92]. Using the UENS as reference standard, Sudoscan has similar operating characteristics as IENFD (AUC of 0.76 v 0.75 for IENFD, p = NS) [92]. Furthermore, in the cross-sectional cohort of healthy controls and diabetes individuals, Sudoscan™ demonstrated a moderate but significant correlation with sural serve amplitude ($r = 0.34$, $p < 0.02$) [92].

Quantitative sensory tests (QST) for thermal, pain, and vibration perception. These tests are designed to provide a quantitative measure of sensation. They have been shown to provide valuable information for assessing DSPN. Both the San Antonio consensus [93] and the Toronto consensus recommended the use of quantitative sensory tests with thermal thresholds for DSPN diagnosis [9]. Various techniques and devices are available, ranging from the handheld Tiptherm device [94] across current perception threshold devices to the sophisticated computerized instruments such as CASE IV from WR Medical® and the NeuroSensory Analyzer (TSA) from Medoc® [23, 95]. The latter two have the advantage of a large database of accurate normative values.

A drawback of QST is the psychophysical subjective nature, requiring cooperation from the patient. This has resulted in a wide variation of published reproducibility values [30, 96]. Abnormal results suggest a dysfunction somewhere along the sensory pathway, not necessarily directly at the site of testing [96]. However, with good training and standardization of testing methodology, the German Research Network on Neuropathic Pain (DFNS) has shown good test/retest results, enabling inter-observer reliability [97]. Furthermore, QST may have an important role in the quantification of positive sensory symptoms such as allodynia and hyperalgesia [98]. In their recent consensus document, the Neuropathic Pain Special Interest Group of the International Association for the Study of Pain (NeuPSIG) recommended the use of QST for screening for small and large fiber neuropathies, monitoring of somatosensory deficits, and monitoring of evoked pains, allodynia, and hyperalgesia [99]. The group also suggested using QST as the sole test for diagnosis of neuropathic pain. They further highlighted the importance of standardized stimuli and instructions, validated testing algorithms, and reference values [99]. In the 2005 AAN document, the consensus committee noticed too much inconsistency among the studies describing the accuracy of QST, and did not include the methodology in the final case definition of DSPN [8].

Simple handheld vibration perception threshold measurement devices (e.g. neurothesiometer) correlate well with NCS parameters, are quick, reproducible, and painless [100]. Indeed, in some studies, they have shown superior performance to CASE IV (sensitivity for DSPN 70% vs. 49% for CASE IV) [101], and have been validated to predict risk of foot ulceration [102]. Current perception thresholds (Neurometer®) and contact heat-evoked potentials (CHEPS) are new emerging tools with data on reproducibility and its relationship to more established reference parameters [103-104]. The recently developed normative values for CHEPS may allow for wide adoption in research studies [105].

Microneurography. Microneurography is a minimally invasive technique, which allows single-fiber recordings from peripheral axons in conscious subjects [106]. The slow recovery of sodium channels during the relative refractory period shows up as a period of pronounced slowing of conduction velocity, a phase that is more pronounced in C-fibers [107]. Microneurography of C-fibers utilizes this period to judge responsiveness of electrically driven C-units to additional natural stimulation of their receptive fields [107]. Pain from stimulation of cutaneous nociceptive C-fibers and Aδ fibers is felt as superficial pricking or burning in the skin, and is projected with an accuracy of 1-2 cm relative to the receptive fields of the stimulated fiber [107-108]. The technique also allows for functional classification of the C-fibers into mechano-sensitive, mechano-insensitive nociceptors, or sympathetic fibers [106, 109].

There is emergent data on differences in C-fiber subtype ratios between the young and aged human, but broad age- and gender-specific normative values are lacking [106]. Furthermore, validation of the technique with other more accepted markers

of DSPN is limited. The finding that specific small nerve fibers play a role in positive symptom generation is exciting. Therefore, microneurography is likely to have a future role as an objective measure of pain and an endpoint in pain pharmacotherapy research. However, microneurography can be time-consuming and difficult, requiring a patient subject and an expert investigator.

Tests for visceral autonomic neuropathy. Our review has concentrated on somatic DSPN, but the importance of diabetic autonomic neuropathy cannot be further emphasized as they are essentially small nerve fibers. Prevalence data vary according to the criterion used, but have been reported to be between 2% and 65% in the cohorts studied, increasing with age and diabetes duration [110]. Evidence of the concomitant presence of cutaneous, cardiac, and visceral autonomic neuropathy in DSPN has been reported [111-112]. Importantly, there is a strong association between cardiac autonomic neuropathy and cardiovascular mortality [2]. Heart rate variability (HRV) employing interbeat (RR) intervals of the electrocardiogram is by far the most used technique with a reported specificity of 80% [9]. In the quest for variability, this method can be performed by applying the deep breathing technique, valsalva manoeuver, or lying-standing [110]. HRV is an early symptom of cardiac autonomic neuropathy [113]. Orthostatic hypotension is another easily measurable parameter that is defined as a fall in blood pressure >30 mm Hg systolic or >10 mm Hg diastolic blood pressure in response to a postural change, usually from supine to standing; it is another recommended method in the Toronto Consensus [110]. Other tests recommended in the Toronto consensus include measurement of baroreceptor sensitivity, muscle sympathetic nerve activity assessment, measurement of plasma levels of catecholamines, and cardiac sympathetic scintigraphic mapping [9]. There is increasing recognition that visceral autonomic neuropathy may be less frequent than skin/sudomotor autonomic functions or lag behind in DSPN, while skin/sudomotor autonomic functions are frequently impaired [111, 114].

3.3 Methods for assessment of large fiber neuropathy

Routine use of 10 gm monofilament (MF) or a 128 Hz tuning fork is advocated in busy diabetic clinics to confirm the presence of DSPN [115]. However, these devices only detect advanced DSPN. The MF was developed more as a marker for loss of protective sensation, and is a good predictive tool for risk of foot ulceration [116]. In this review, we focus on new bedside tests for large fiber neuropathy, while providing a brief overview of electrodiagnostic improvements.

Electrophysiological studies. Large nerve fibers (A-alpha, A-beta, and A-gamma) mediate touch, vibration, and proprioception, and also innervate muscle spindles; established abnormalities may be detected by clinical examination. For more precise characterization, electrodiagnostic studies of nerve conduction parameters remain the benchmark for the diagnosis of DSPN (and atypical neuropathy). Some researchers consider them an extension of clinical neurological examination [21]. Nerve conduction studies are sensitive, specific, reproducible, and validated measures of DSPN, with the ability to differentiate established distal, axonal, and sensory changes of DSPN from proximal motor demyelination or demyelination causes [117].

Initial studies looking into nerve conduction and nerve morphometry suggested that segmental demyelination along with axon loss was the hallmark of diabetic neuropathy [118-119]. However, Dyck *et al*. latterly concluded from their studies that segmental demyelination was secondary to axonal degeneration [120-121]. The most distal sensory nerves (sural, plantar) typically provide the first electrodiagnostic evidence of DSPN [21]. Subsequently, progressive changes may develop in the distal sensory and motor nerves and also in upper limb nerves. In the well characterized population of the Rochester Diabetic Neuropathy Study, the most frequent abnormal attributes under the 2.5th and over 97.5th percentile were fibular (peroneal) motor nerve conduction velocity (26.3%), sural sensory nerve action potential (25.4%), tibial MNCV (24.8%), ulnar MNCV (21.3%), fibular F-wave latency (16.9%), and ulnar F latency (16.0%) [122].

The AAN consensus from 2005 suggested a simplified NCS protocol for detection of DSPN. Sural sensory and peroneal motor NCS acquisitions were considered most sensitive, and were recommended as the first line tests. If they remained normal, no further electrodiagnostic studies were recommended [8]. If abnormalities were present, the consensus was to include ulnar and median sensory NCS along with median motor values, and to assess the contralateral limb parameters [8]. Furthermore, electrodiagnostic studies, especially motor conduction velocity, continue

to be FDA recommended surrogate endpoints in epidemiologic and neuropathic drug development trials [123].

Muscle pains are common in neuropathy, and 'cramps' have been reported in up to 33% of such individuals [124]. These symptoms are in accordance with peripheral nerve hyper-excitability. Although not routinely used in neurophysiology laboratories, assessment of peripheral nerve hyper-excitability using slow repetitive nerve stimulation (to assess cramp after discharges) is a neurophysiological technique with reported sensitivity of 79% and specificity of 88% [125-126]. F-wave latency may serve as a sensitive indicator of DSPN [127-128]. However, its role in diagnosis and characterization of DSPN remains unclear, and both the AAN consensus and Toronto consensus do not provide specific recommendations on its use.

Features suggestive of demyelination, including significant reduction in motor conduction velocity and prolonged distal motor latency, may be associated with DSPN in some patients. This makes it difficult to differentiate DSPN from the immunologically mediated chronic inflammatory demyelinating polyneuropathy [129]. Electromyography (EMG), the needle electrode examination of muscles, supplements the NCS, but has a limited role in DSPN. Typically, an EMG would be performed to investigate possible other diagnoses in addition to DSPN such as radiculopathy, inflammatory myopathy, or atypical motor neuropathy.

Limitations of electrodiagnostic studies include the need for referral to a neurophysiology lab, the 30-40 minutes testing time, and a degree of patient discomfort. More recently, the NPhys Trial 3 demonstrated that NCS attributes were without significant intra-observer differences, but there were significant inter-observer differences, sometimes within the same neurology department [130]. Robust normative values will help to characterize the presence of DSPN with greater accuracy. Studies have shown a significant effect of age, gender, type of diabetes, and anthropometric measures on NCS [131-132]. Inter-observer variation in NCS acquisition may be reduced with the use of detailed standard reference values and a clearly pre-defined percentile level of abnormality [130]. With recent studies, demonstrating SFN to precede NCS changes and small fibers to possess the ability to regenerate (albeit for temporary periods), the use of NCS measures as preferred endpoints by regulatory authorities is being increasingly debated.

NC-stat®/DPNCheck™. The NC-stat®/DPN Check™ system (Neurometrix, Waltham, MA) is a simple, low-cost, handheld point-of-care device that measures NCS quickly and accurately with basic training, but without the need for laboratory-based, expensive electrodiagnostic equipment. The system consists of a single-use flexible biosensor panel, a handheld device with LED display, software that can download data from the device, and a cable to connect with a computer. It provides information on two NCS measures: 1) sural nerve conduction velocity (SNCV) and 2) sensory nerve action potential (SNAP) amplitude. Previous studies have shown that it has excellent correlations with conventional NCS ($r = 0.76$ to 0.91, $p < 0.001$) and excellent intra-rater reliability inter-class coefficients of 0.97 and 0.94 for SNAP and SNCV, respectively [133-135]. It has recently been validated in a study comparing its performance in detecting DSPN with that of the LDIflare technique, the latter measuring small fiber function. The NC-stat®/DPNCheck™ system demonstrated an excellent performance (AUC 0.74 to detect DSPN with NDS > 3) [70]. Although the device has a few limitations (sensory amplitude below 1.5 μV being assigned zero and orthrodromic stimulation of sural nerve), it may have a future role in large cross-sectional studies of newly diagnosed diabetes cohorts, and possibly even regular DSPN screening.

VibraTip™. The VibraTip™ is a small key fob-shaped device intended for DSPN screening during routine annual diabetes checks [136]. Whist in principle it is an electronic proxy of a tuning fork, it differs in that it provides near-silent vibration of constant amplitude [136]. The VibraTip™ device is applied to the tip of the halluces, and provides categorical (qualitative) data on vibration perception. In a cross-sectional diagnostic accuracy study, with quantitative vibration perception threshold and NDS as the gold standards, VibraTip™ had good agreement with the reference tests [136-137]. Relative to the Neurothesiometer, VibraTip™ has a reported sensitivity of 79-100% and specificity of 83-97% [137-139]. It has not yet been fully evaluated against NCS or SFN measures. The VibraTip™ was recently evaluated by the National Institute for Health and Care Excellence (NICE) for the purpose of clinical use in the detection of DSPN. The final recommendation was that, whilst the technology demonstrated promise, submitted and published evidence was insufficient to determine diagnostic superiority or equivalence of VibraTip™ confidently compared with the MF or the 128 Hz tuning fork [136]. Additionally, the committee opined, the device is unlikely to reduce foot examination costs [136].

Table 6. Selection of the normative data available

Study by	Year	Modality	Site	Number of subjects	No. of decades in sample	Percentage change/decade	Relationship with age
Bianchi *et al.* [39]	2004	IENFD measurement	Distal leg	87	6	7.0%	r = -0.46
Umapathi *et al.* [155]	2006	IENFD measurement	Ankle	84	6	5.8%	r = -0.46 ($p < 0.001$) at ankle for age, no gender difference
Bakkers *et al.* [156]	2009	IENFD measurement	10 cm above lateral malleolus	188	6	5.9%	r not reported, but inverse significant correlation, men lower densities
Lauria *et al.* [39]	2010	IENFD measurement	10 cm above lateral malleolus	550	6	6%	Relationship with age and gender. r not reported, but $p < 0.0001$ in women and $p = 0.002$ in men
Yarnitsky [147]	1994	Thermal thresholds	Dorsal foot method of levels	106	6	CDT 0.35% WDT 0.62%	No relationship with age
Seah *et al.* [157]	2007	Cold detection thresholds	Non-dominant hand		4.5 (20-65 years)	2%	Higher in males but no age relationship
Vas *et al.* [32]	2013	LDIflare	Dorsal foot	94	6	5.5%	r = -0.42, $p < 0.0001$
Niederer *et al.* [158]	2007	Corneal confocal microscopy	Cornea	85	Not divided into age groups	9.0%	r = -0.43, $p < 0.0001$
Wu *et al.* [159]	2012	Corneal confocal microscopy	Cornea	65	Not divided into age groups	-	No association with age noted. β coefficient rather than Pearson's correlation used
Tavakoli *et al.* [146]	2015	Corneal confocal microscopy	Cornea	343		CNFL decreased in men (-0.045 mm/mm^2 per year), women (-0.060 mm/mm^2 per year)	Relationship with age and gender

Legend: CDT – cold detection threshold; CNFL – corneal nerve fiber length; IENFD – intraepidermal nerve fiber density; LDIflare – laser Doppler imager flare; WDT – warm detection threshold.

Ipswich Touch Test. The Ipswich Touch test (IpTT) was developed out of a need for a 'ready-at-hand' neuropathy screening tool for the detection of individuals with the highest risk of foot ulceration, with the specific aim of reducing hospital-acquired foot ulceration [140]. Relative to the vibration perception threshold (VPT), it has good sensitivity (77%) and specificity (90%), which is not significantly different from the established 10 gm monofilament technique (sensitivity (85%) and specificity (88%) when compared with the VPT). Also, it is straightforward enough to be used by relatives, healthcare staff, and friends, thus supporting self-examination and providing patient education [141]. Bowling *et al*. have also independently validated the IpTT, demonstrating that it produces identical results to Vibratip™ (kappa, $\kappa = 1.0$), with almost perfect agreement when compared with the VPT ($\kappa = 0.97$, $p < 0.001$) and the Neuropathy Disability Score ($\kappa = 0.92$, $p < 0.001$) [137]. More recently, a group from Saudi Arabia has evaluated the IpTT as a reliable screening tool for the loss of protective sensation with the ability to overcome barriers in foot risk screening [142]. The IpTT has been recommended in the recently published 'How to do a 3-minute diabetic foot exam' as a screening test for detecting sensory loss [143]. This has also recently been approved by the American Diabetes Association (Prof. A Boulton, presentation at the ADA Scientific meeting, Boston 2015).

4. Validation of normative values

Normative values are data that characterize, in a pre-defined population, what is usual at a time point or period of time for a specific test or technique [144]. Such data are very useful in diabetic neuropathy as understanding normative change (related to morphology and function) allows for defining the presence or absence of the condition, and provides useful insight into the possible etiopathogenesis. However, in studies designed to obtain normative data, the analytical sample should be rigorously selected from clear predefined criteria; the methodology should be robust and reproducible, and the interpretation of the results should be appropriate [144-145]. Furthermore, when age effects are to be described or when time is an important consideration, then longitudinal study designs may be needed to evaluate potential cohort effects and epoch effects [144].

Both the somatic and autonomic peripheral nervous system change with age [1, 32, 39, 146]. It is therefore important that accurate normative data is determined with modern sensitive techniques developed for the detection of DSPN. An excellent example is the worldwide normative data report by Lauria and colleagues from Europe and North America for IENFD [39]. Apart from age- and gender-specific normative values, the paper also standardizes the protocol used to derive such data. A similar endeavor with the CCM, published recently, has pooled 1,965 images form 343 healthy volunteers across Europe, Australia, and North America, allowing practical and single-protocol-driven use of this technique [146].

While the commercial tests for thermal thresholds and sudomotor testing have established large normative databases [14, 147-149], tests for small fiber function such as LDIflare and CHEPS have recently established age-defined centile charts [32, 105]. There are no current normative values for microneurography. It is important to note that normative data currently available for these select tests are for predominant European/Caucasian cohorts; their application in other ethnicities has not been fully studied. We have summarized a selection of the available normative data for small fiber tests (**Table 6**).

A recent study has shown that the use of age-based normative values may enhance the diagnostic efficacy of the test [32]. However, there exists considerable variation in methodology used by research teams for the same diagnostic technique, an issue that affects normative results and diagnostic accuracy [150].

5. Genetic studies in DSPN

A significant body of new evidence is pointing towards a link between genetic factors and the development of diabetic complications [151]. Studies have reported risk association for the genes coding for factors such as vascular endothelial growth factor (VGEF) in retinopathy, engulfment and cell motility (ELMO1) in nephropathy, and ADIPOQ in coronary artery disease [151]. Candidate gene studies of dysglycemic pathways have uncovered genes coding for aldose reductase activity inhibition (such as AKR1 B1) in the development of diabetic retinopathy and nephropathy [151-152]. Aldose reductase is a key rate-limiting enzyme in the polyol pathway also implicated in the development of diabetic neuropathy.

Studies looking at small fiber neuropathic pain have also established roles of various sodium channels (gain of function SCN9A mutations), while mutations in the vanniloid receptor gene, TRPA1, may lead to episodic familial pain syndromes [31]. Similarly, channelopathies leading to loss of function mutations may cause insensitivity to pain syndromes [153]. Polymorphisms in the adiponectin (ADPN) gene, T45G and G276T, have also recently been associated with increased risk of developing DSPN in type 2 diabetes [154]. The authors concluded that the polymorphisms led to a downregulation of ADPN serum level, an insulin sensitizer and anti-inflammatory agent. However, the reported associations were weak and inconsistent, and no studies predicted a clear relationship of any specific genetic mono- or polymorphism with DSPN. Nevertheless, this is an exciting field of future research, both from a therapeutic and a clinical perspective.

6. Summary

DSPN is a heterogeneous constellation of clinical and subclinical syndromes. The development of modern techniques able to more precisely measure function and structure of small fibers has led to earlier detection and better characterization of this condition. Nevertheless, the field is still in its infancy, and the ability of some of the modalities to demonstrate neuronal regeneration, whilst exciting, needs to be evaluated as a proof of principle.

Furthermore, etiopathogenesis studies are relatively sparse and studies of neuronal plasticity and regeneration, in particular potential differences between the young and aged, are lacking. Large fiber markers are being refined constantly, and the availability of a point-of-care device may reinvent

screening for neuropathy. At the same time, the availability of new methods to detect loss of protective sensation, some of them with no cost implications, enables neuropathy screening to all those with diabetes, in particular in regions of the world with resource limitations.

Disclosures: The authors report no conflict of interests.

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